



Appn No.: 10/027,219
 Applicant(s): Marc Vidal et al.
 REVERSE TWO-HYBRID SYSTEMS

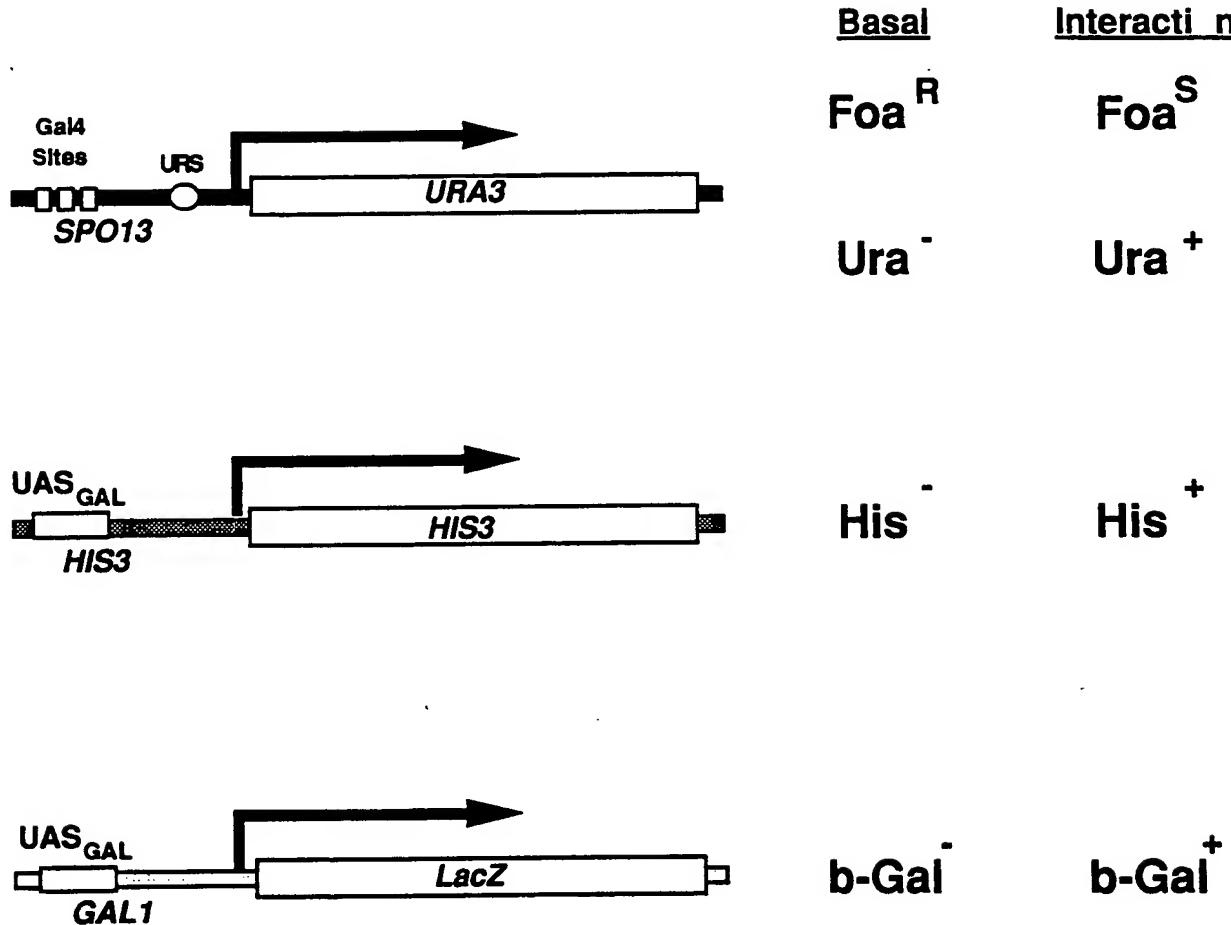


FIG. 1



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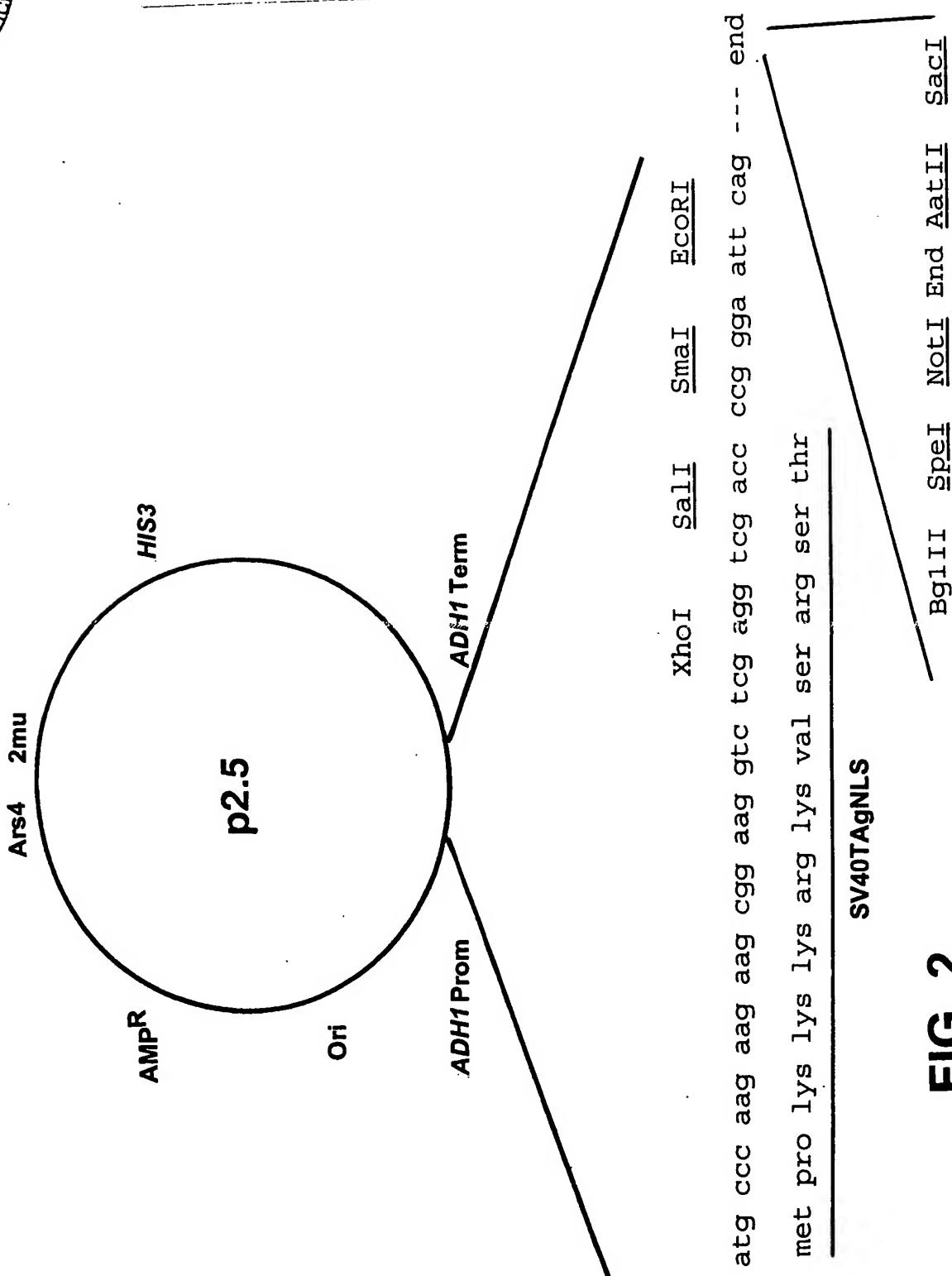
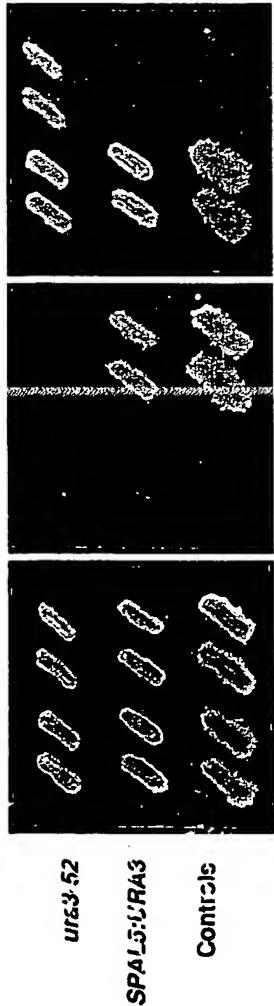


FIG. 2



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DB+AD
Gal4



Sc-L-T Sc-URA Sc-L-T-FOA 0.1%

FIG. 3



Sc-L-T+FOA 0.2%

Sc-L-T

Sc-L-T-URA

FIG. 5

DB AD	Gal4	Vectors
DB-Fos AD	DB-Fos AD-Jun	DB AD-Jun
DB-Rb AD	DB-Rb AD-E2F	DB AD-E2F
Controls		

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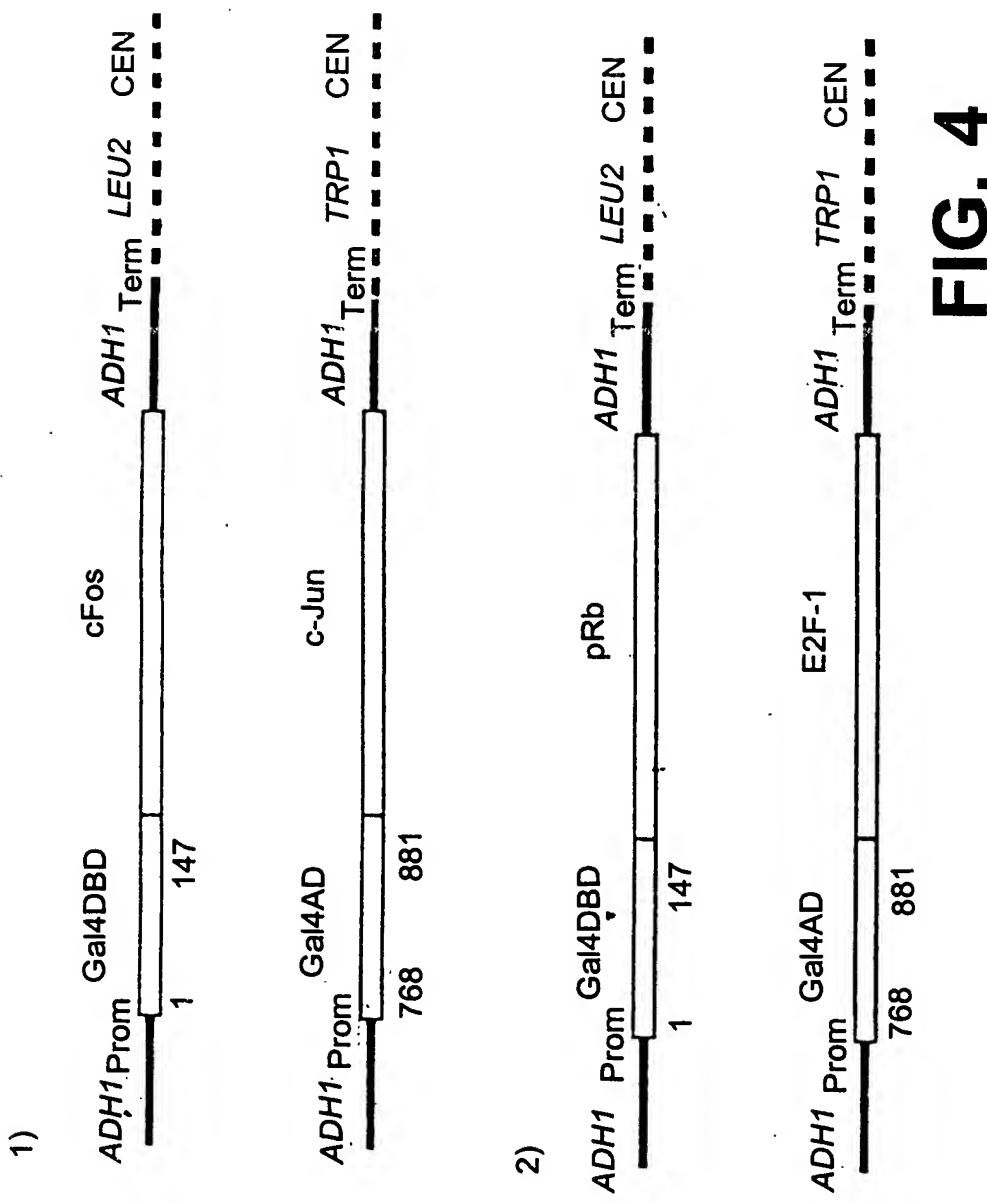
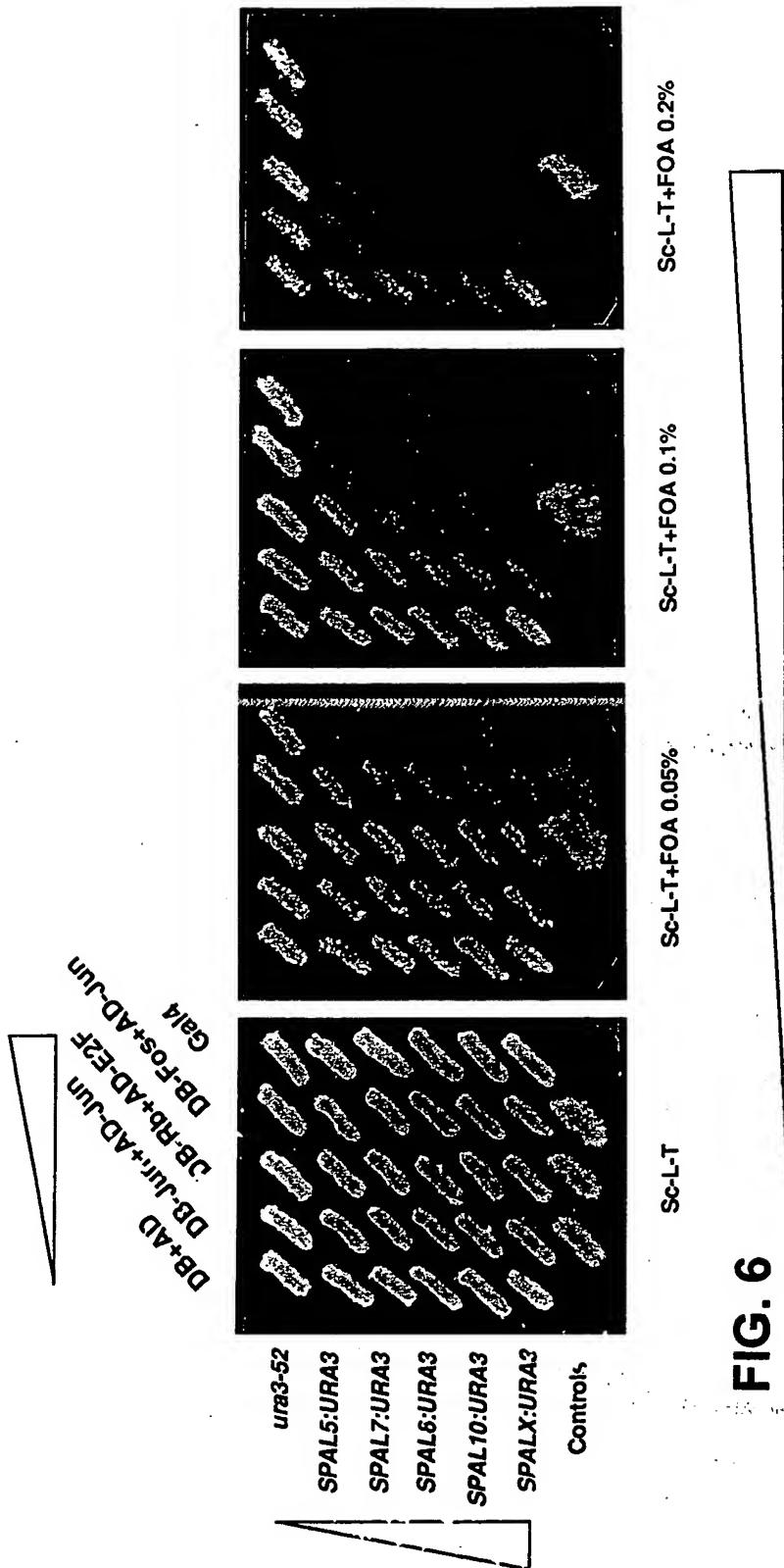


FIG. 4

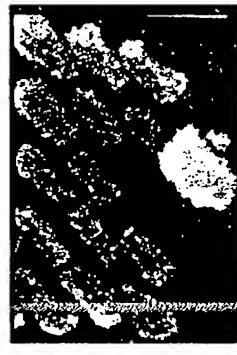




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FIG. 7

DB-Ac
 DB-Rb+AD-E2F
 DB-Rb+AD-E2F
 DB+AD



5-FOA
 DB-Rb+AD-E2F
 DB+AD

Sc + 5-FOA (0.2%)

Sc

Controls

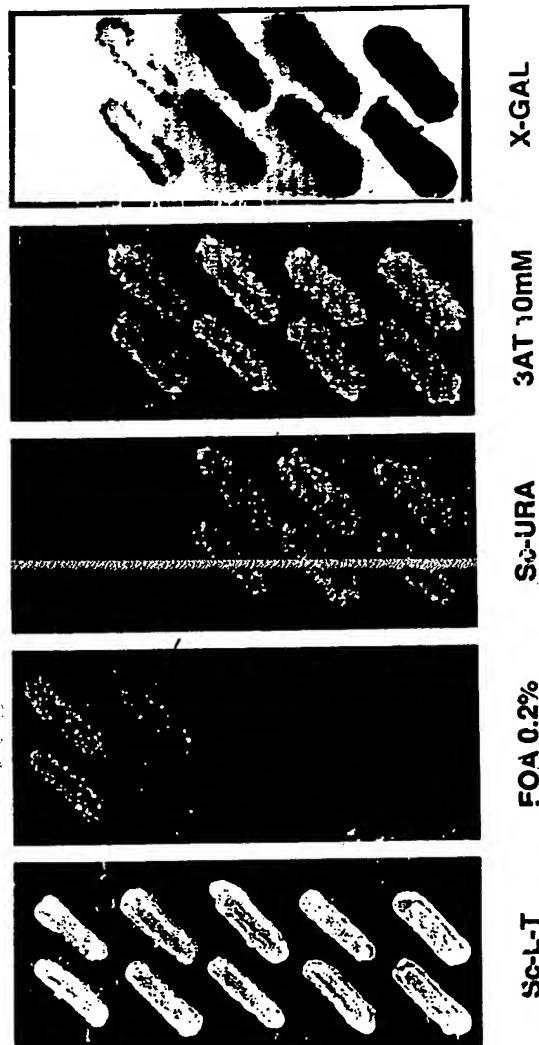


FIG. 8

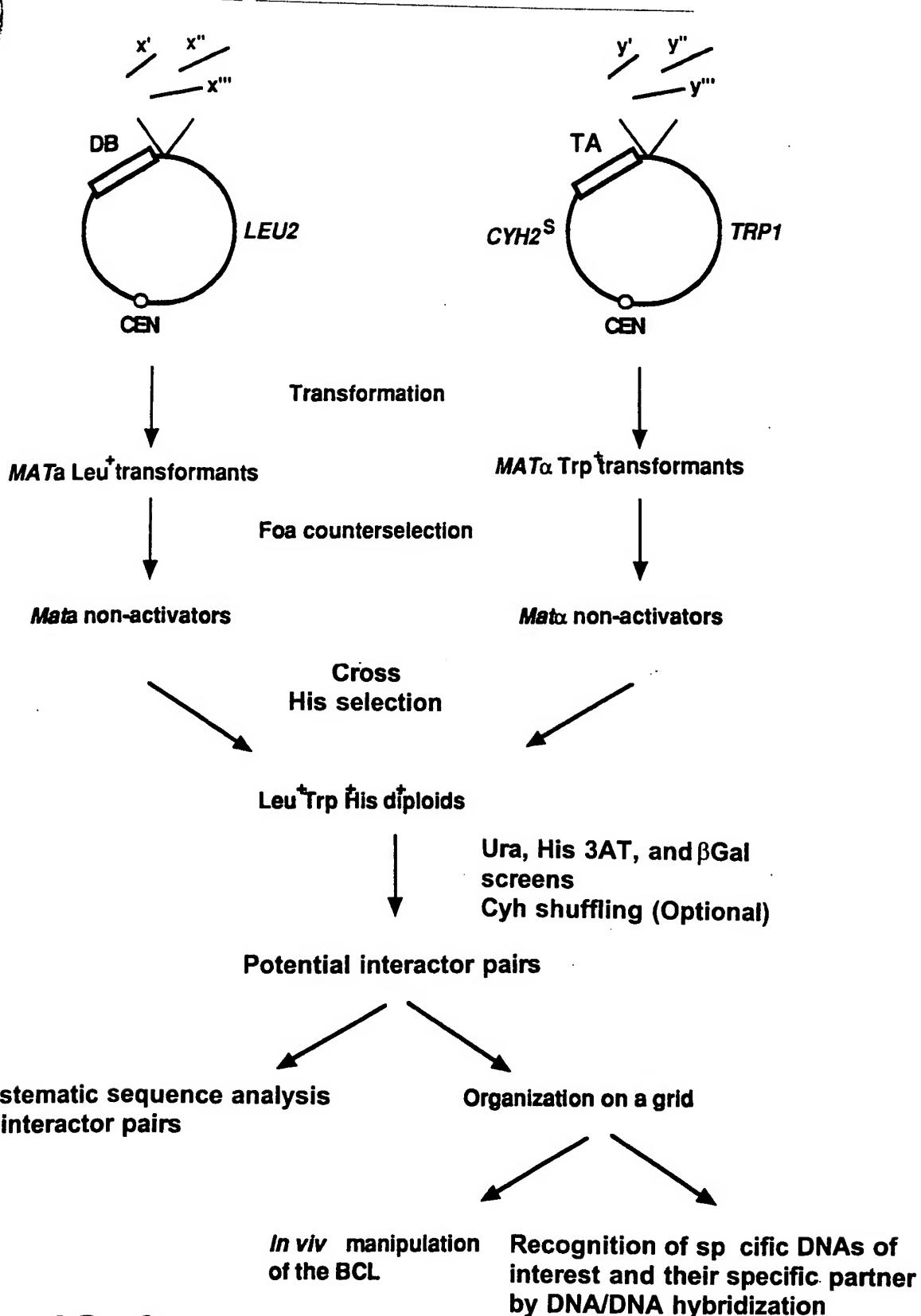
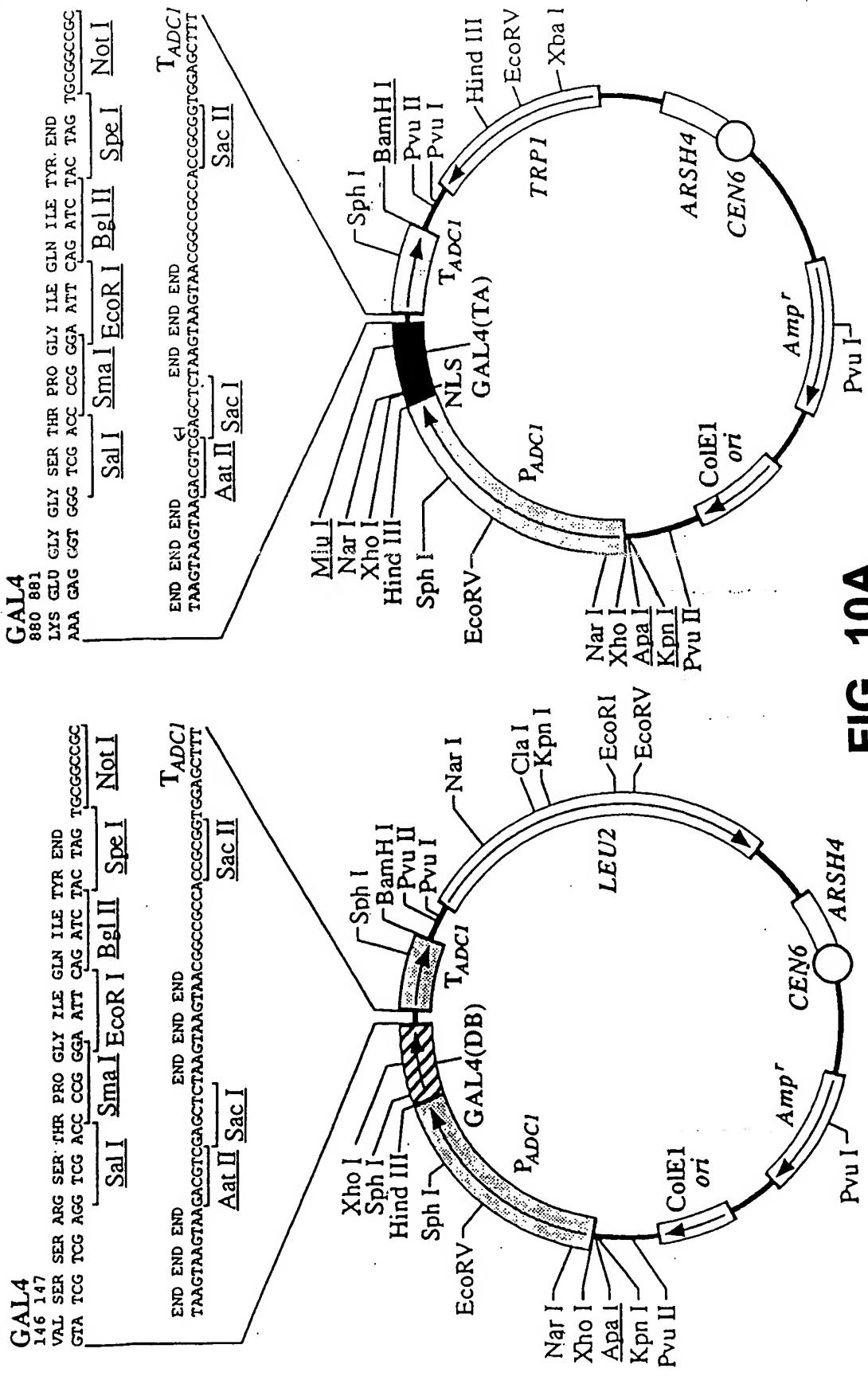


FIG. 9

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**FIG. 10A**



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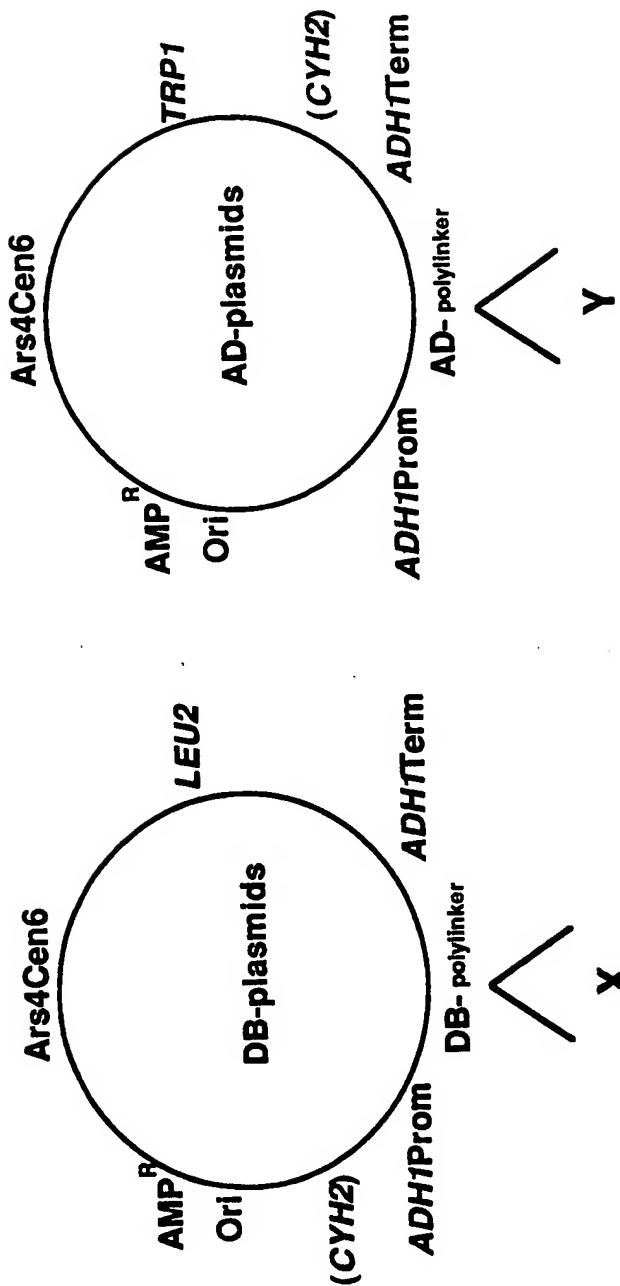


FIG. 10B



DB-X	Known interacting			"Novel" interacting		False positive	
	Total	His+	Retested				
None	1x10	6	1	0			
p130	5x10	5	19	9	0	5 → 2	
DP1	2x10	5	7	7		6 → 2	1 → 1
pRb	1x10	6	20	0			
p35	1x10	6	20	8	0	8 → 2	0
CDK3	1x10	6	38	16			
CDK3	1x10	6	38	16			
DCC1	3x10	6	81	23	0		
Z bu	1x10	6	81	23			

FIG. 11



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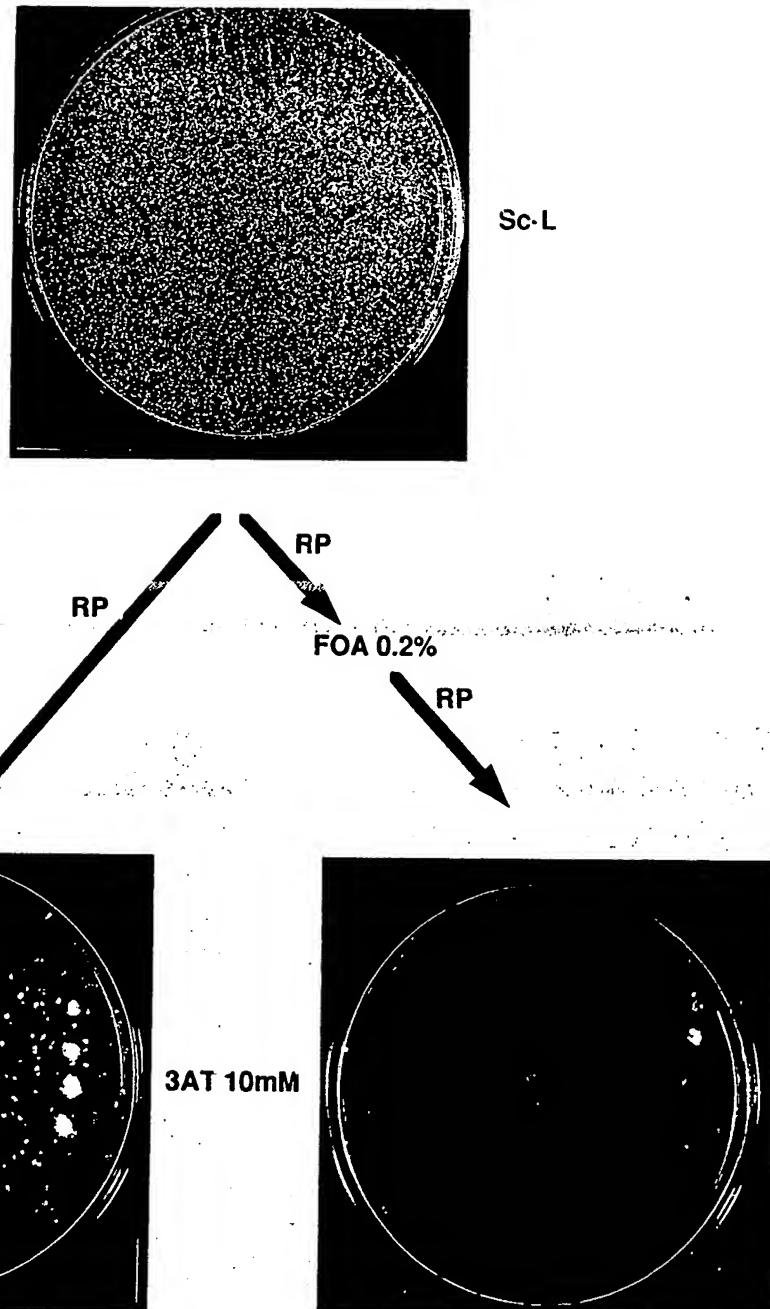


FIG. 12

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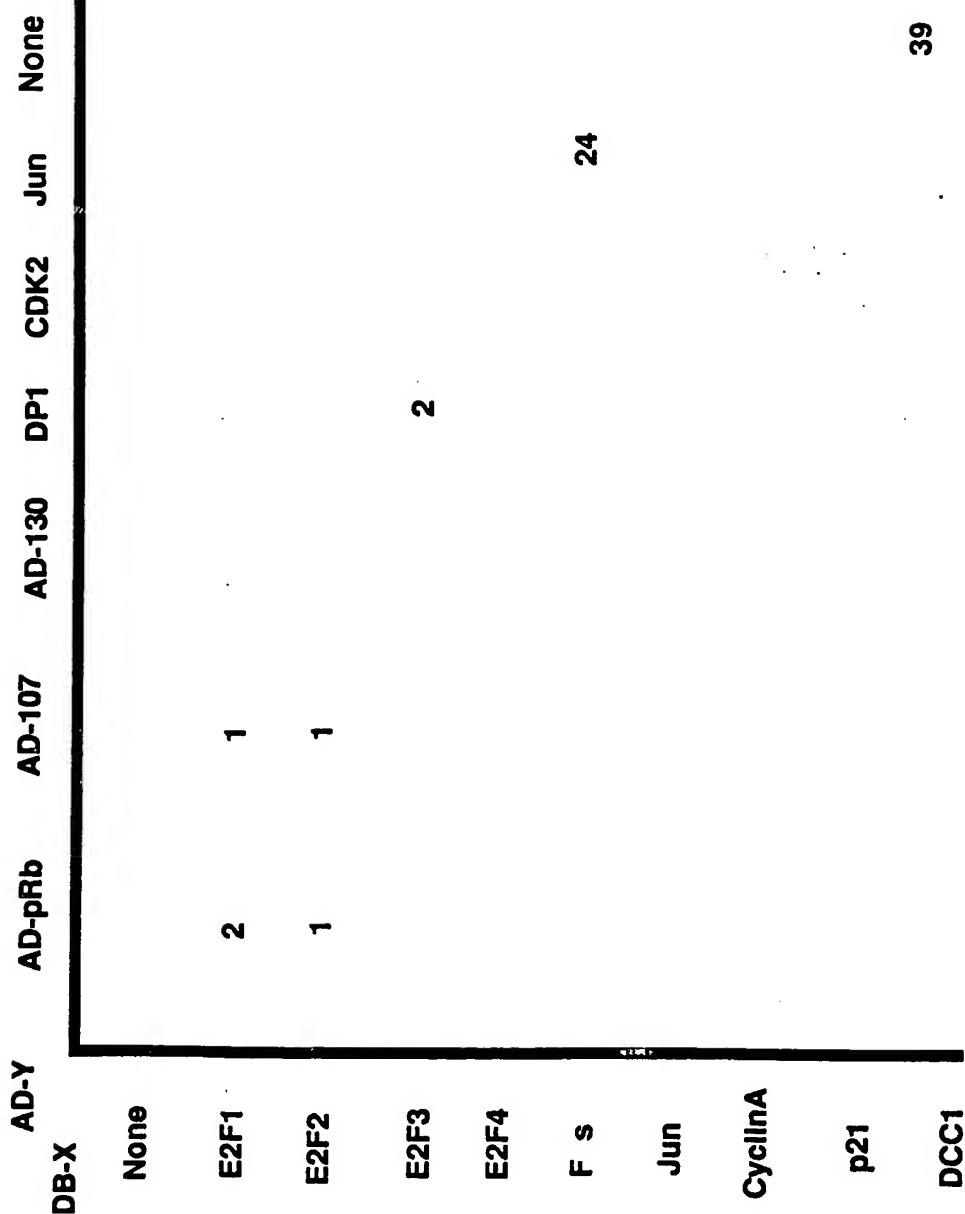


FIG. 13

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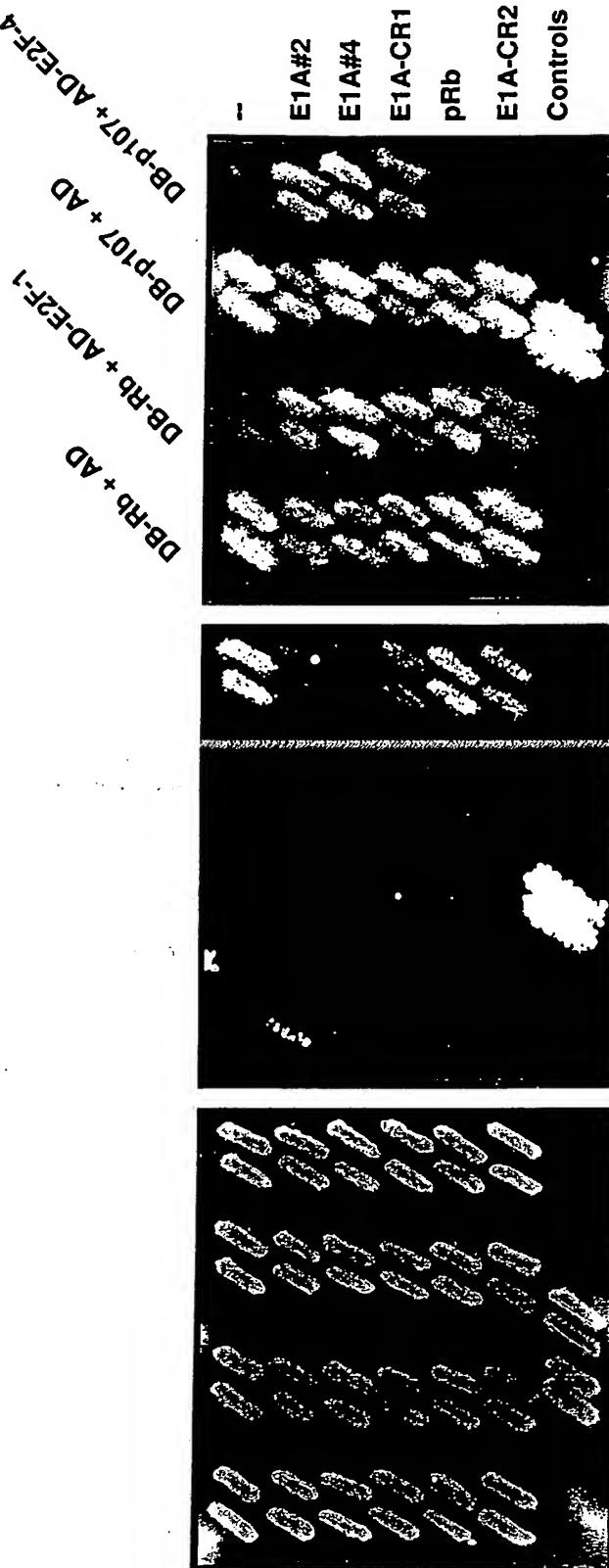
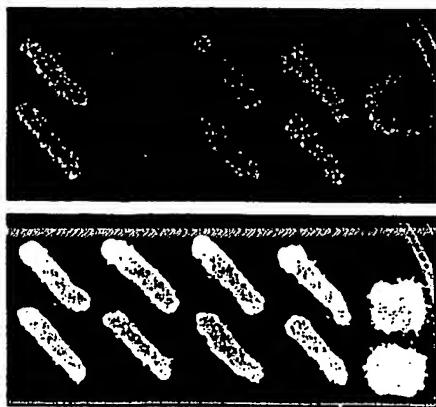


FIG. 14

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 REVERSE T. / S-H. GRID SYSTEMS



FIG. 15



Controls

DB+AD

DB-Rb+AD-E2F1

DB-F1, AD-E2F1-3C

Sc-L-T Sc-L-T+FOA 0.2%

JB-DP1 +
 AD-E2F1-20
 AD-E2F1-21
 AD-E2F1-32
 AD-E2F1-34
 AD-E2F1
 Controls 1,2,3,4

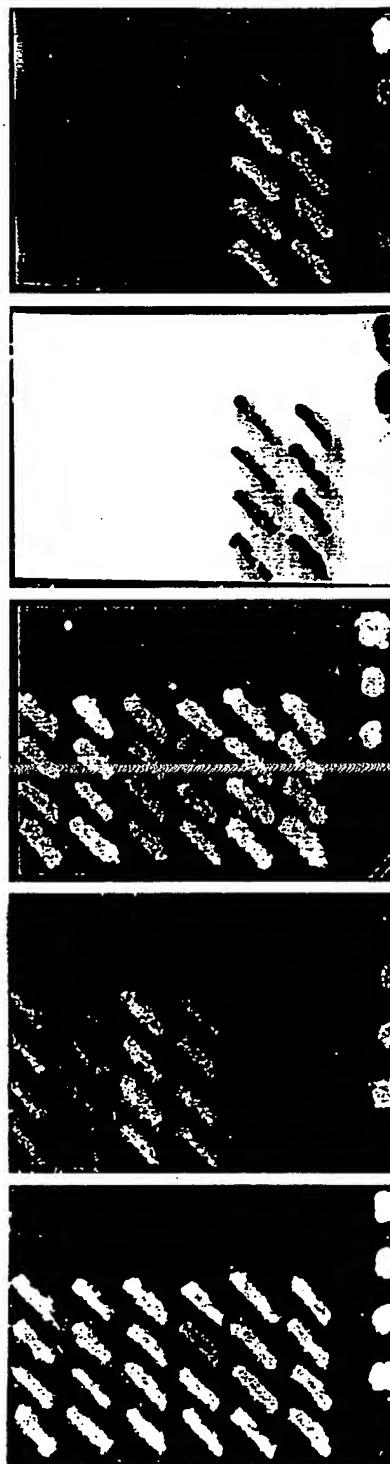


FIG. 20

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 INVERSE TWO-HYBRID SYSTEMS

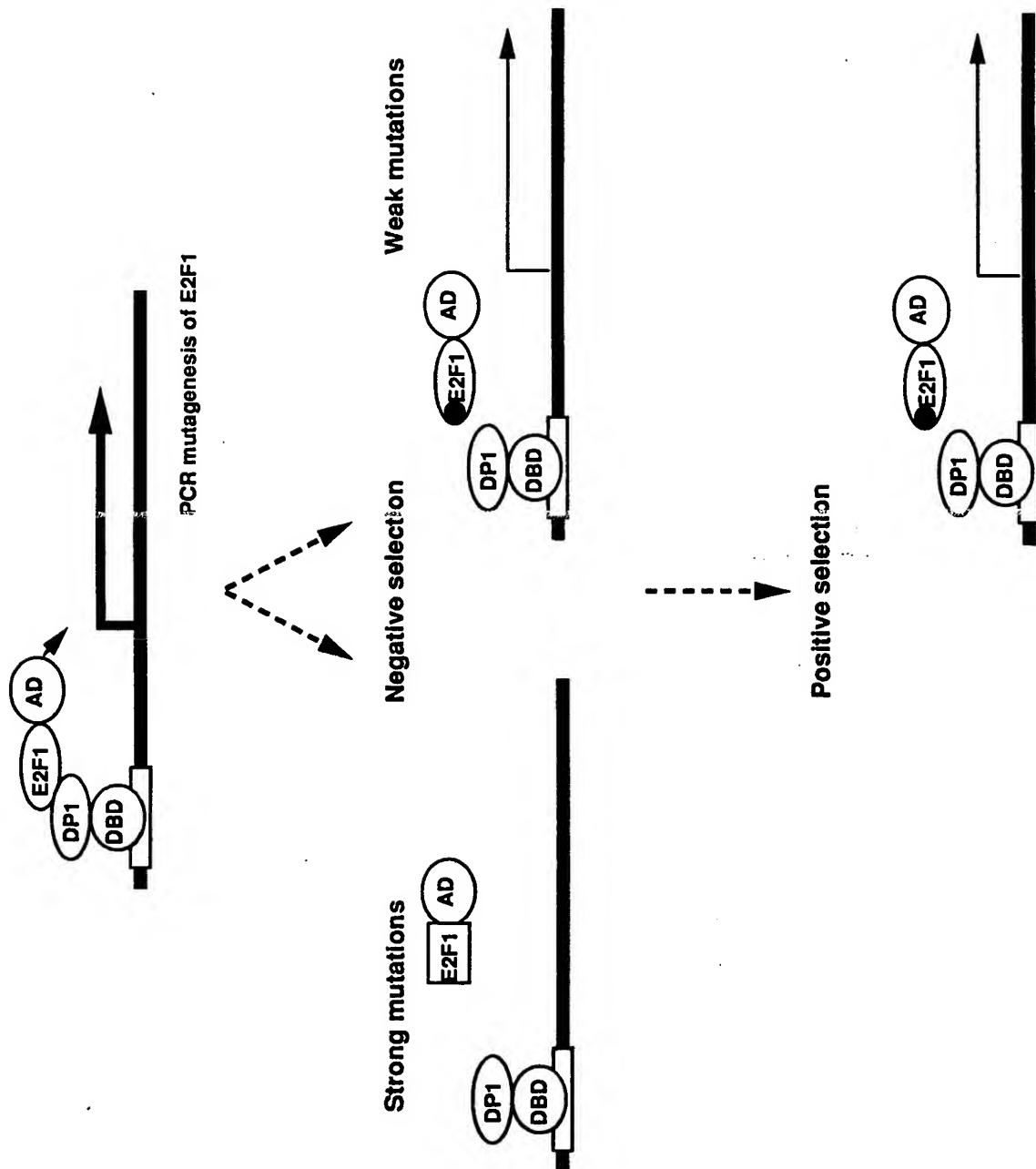


FIG. 16

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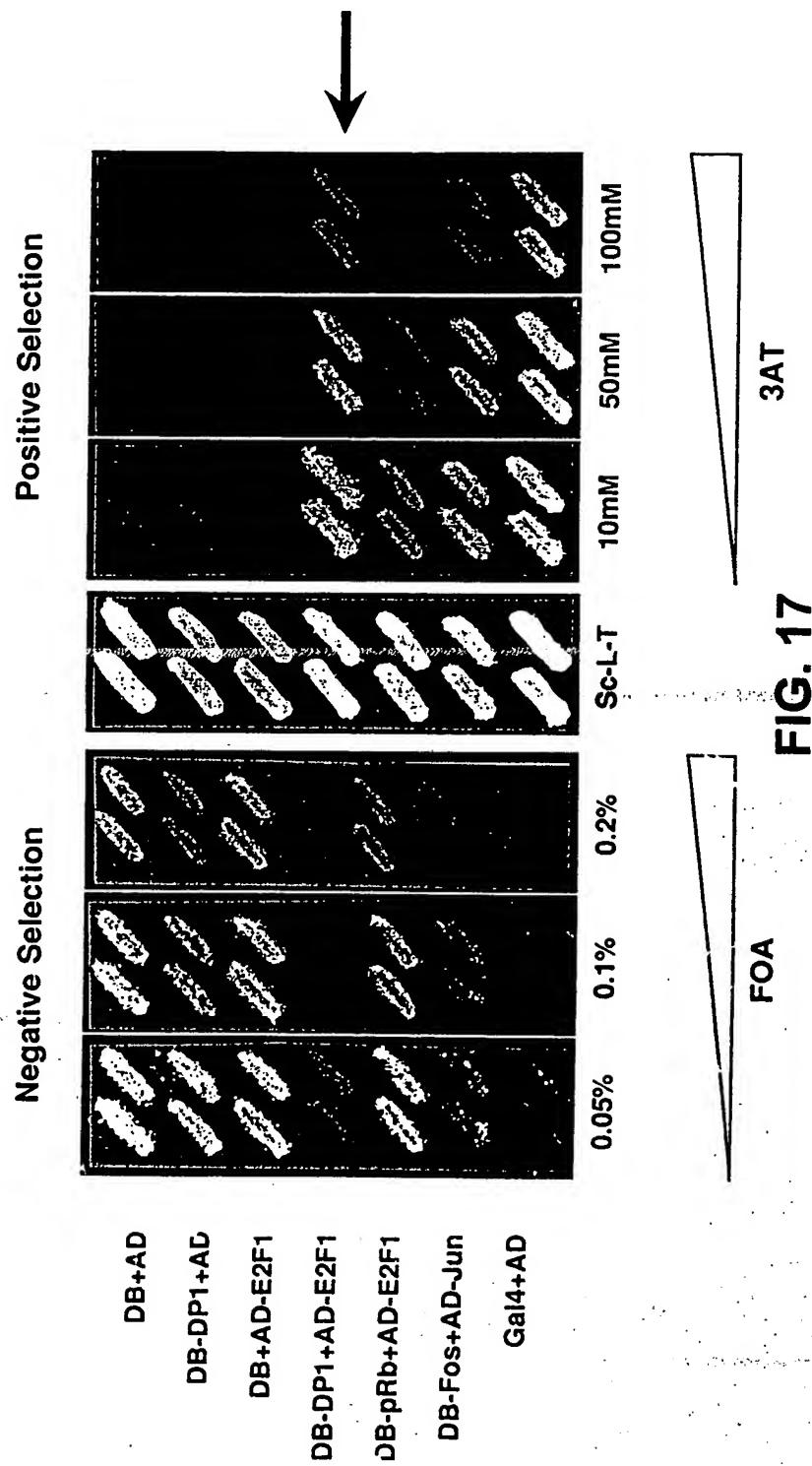


FIG. 17

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PCR reaction

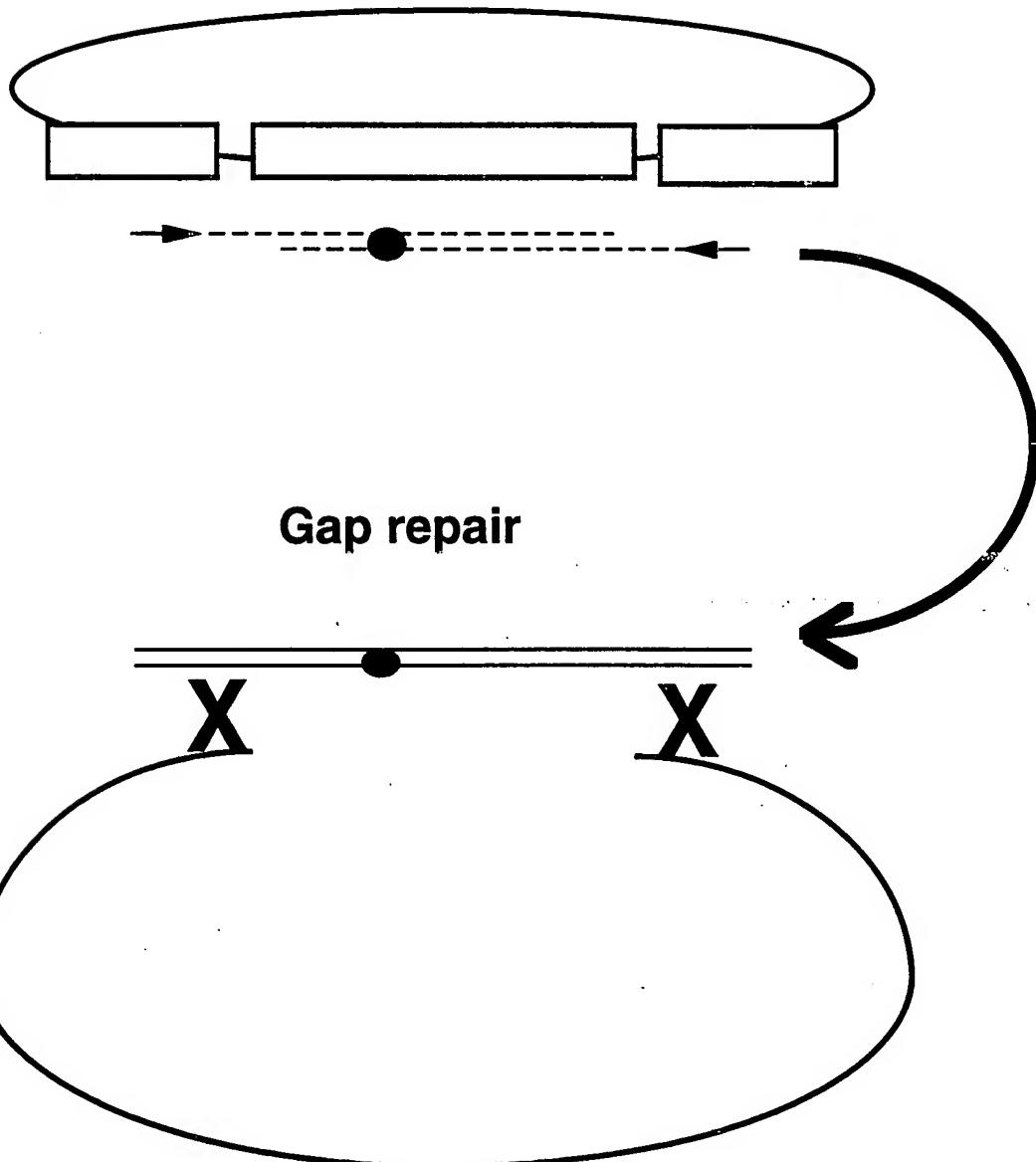


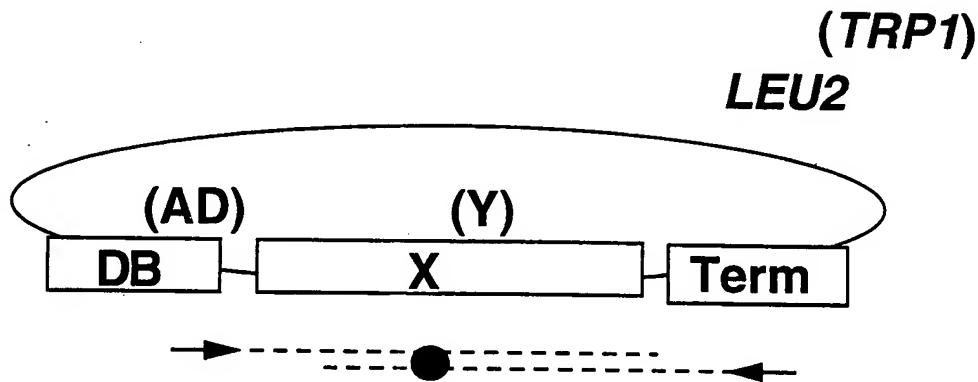
FIG. 18A



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In vitro mutagenic PCR reaction



In vivo gap repair

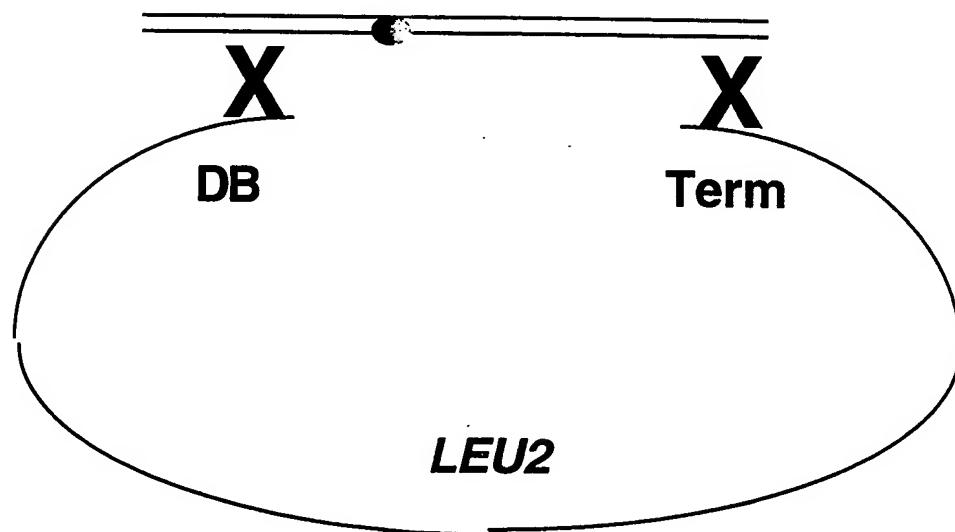


FIG. 18B

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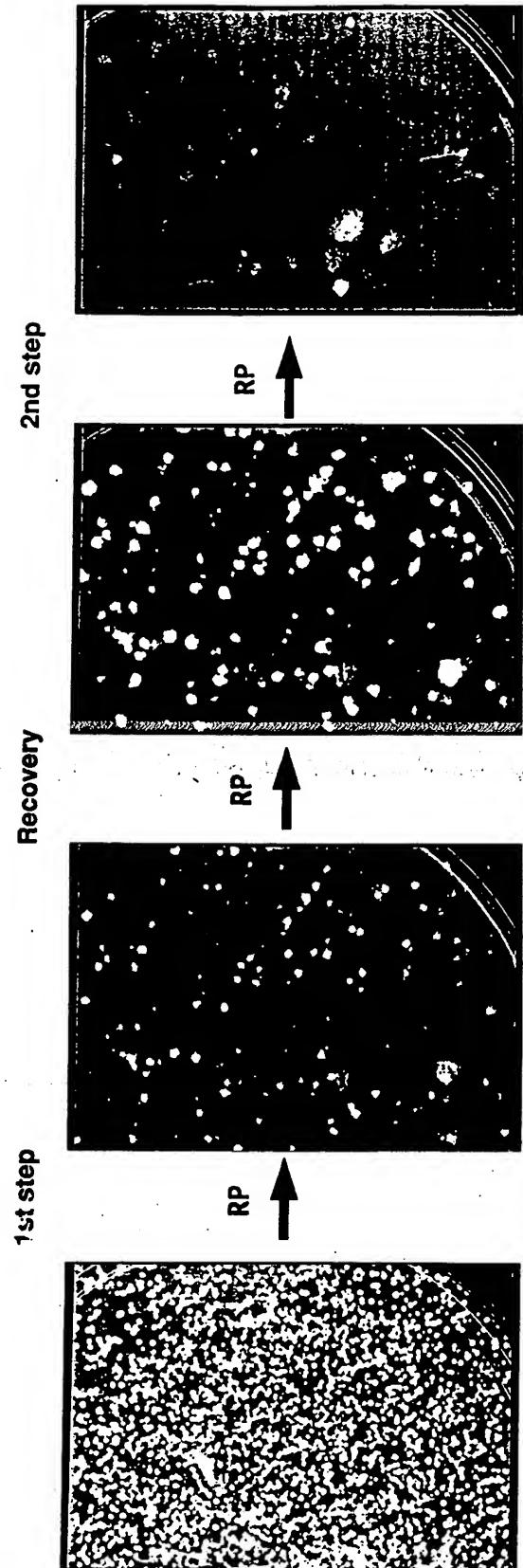


FIG. 19

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MARKED BOX 2

283	Q	I	N	K	E2F5
	Q	I	H	K	E2F4
	Q	I	H	P	E2F3
	Q	I	Y	P	E2F2
	Q	I	F	P	E2F1

					E2F1-20
					E2F1-30
					E2F1-32
					E2F1-31
					E2F1-65

FIG. 21



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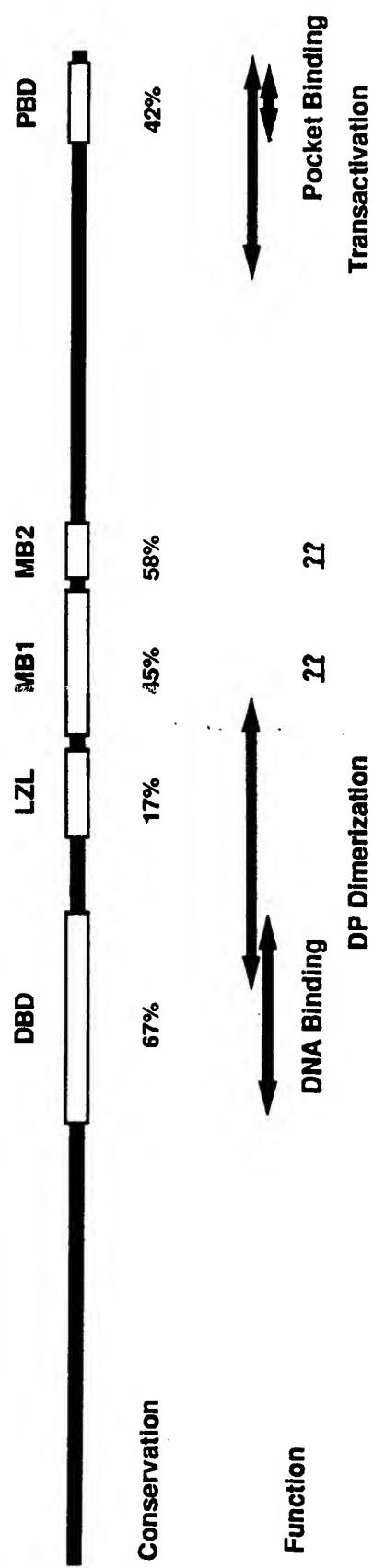


FIG. 22

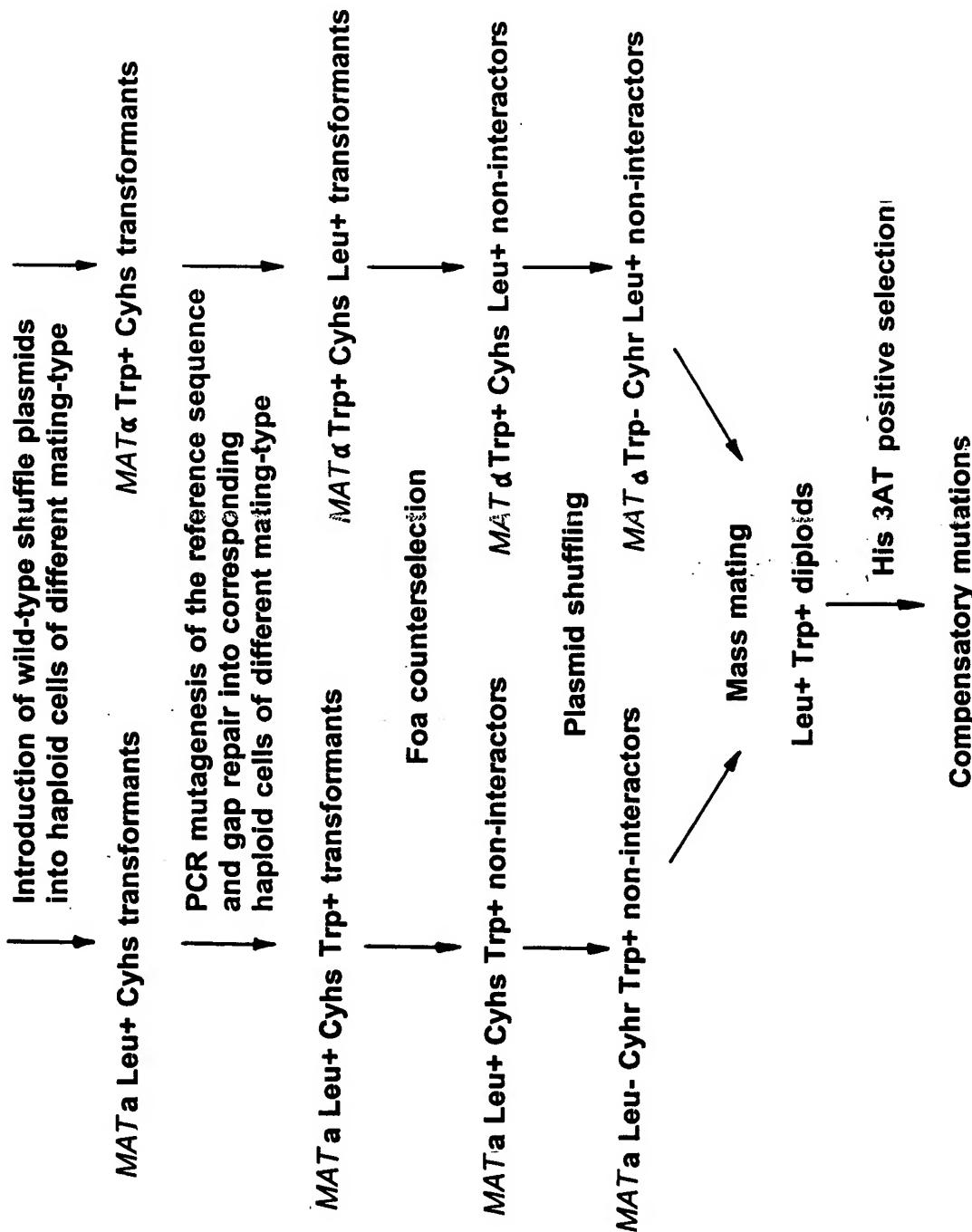
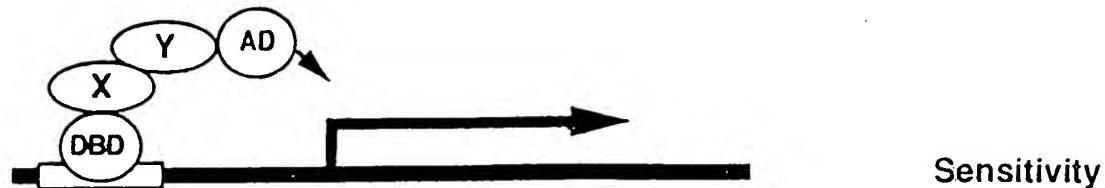


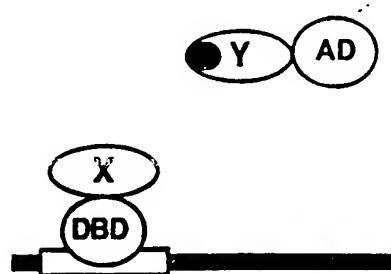
FIG. 23A



Sensitivity

Mutagenesis of X (or Y) partner

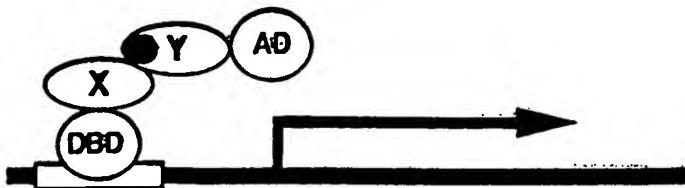
SELECTION for Foa Resistance



(Restrictive conditions)

Replica-plating

SELECTION for Ura/His growth



(Permissive conditions)

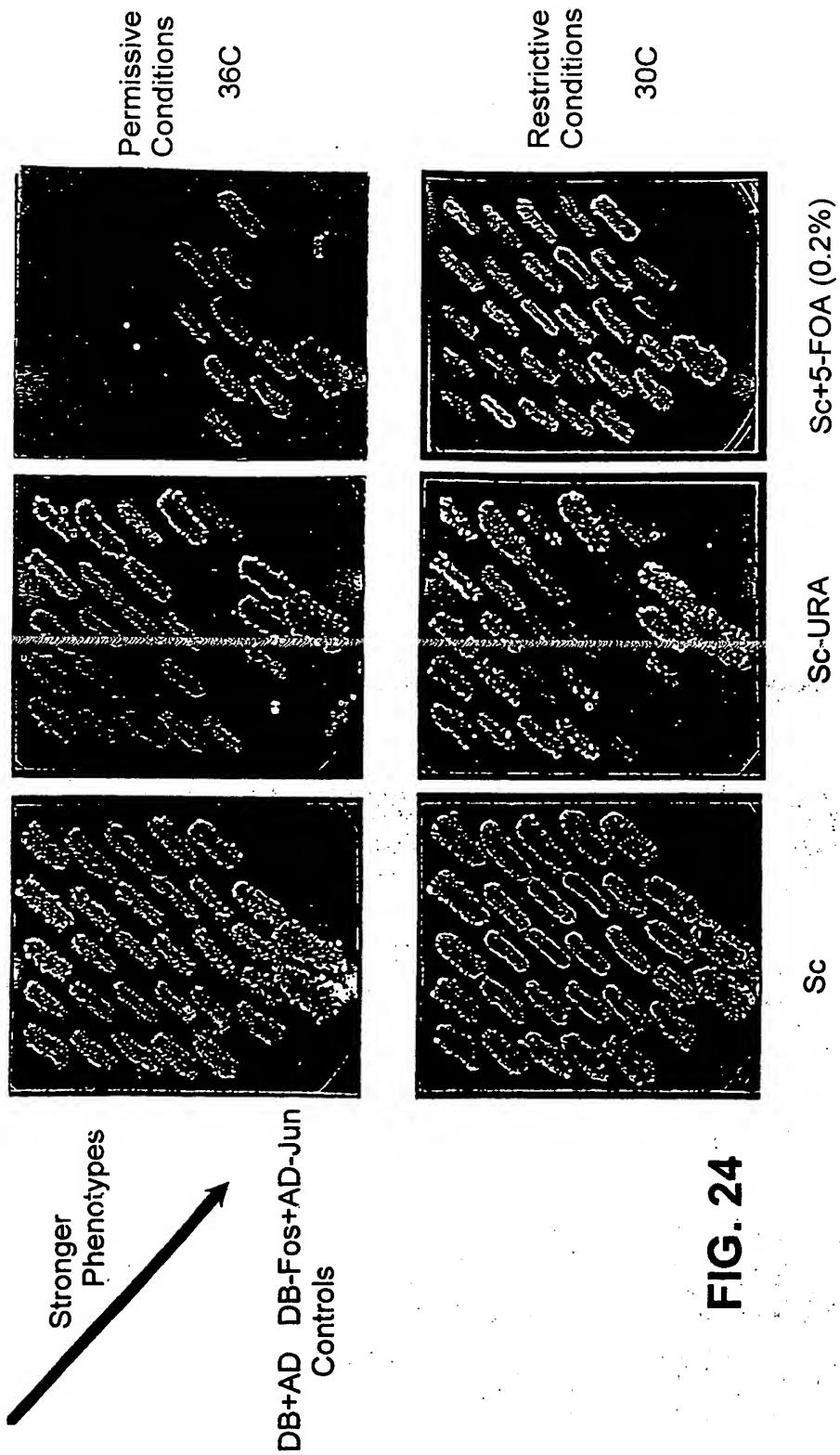
FIG. 23B



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 REVERSE TWO-HYBRID SYSTEMS



CLONE AND EXPRESS
 DB/Ag FUSION

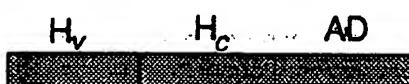
Ag

IMMUNIZE
 ANIMAL



PERIPHERAL
 B CELLS

1. PCR Light and Heavy Chain Variable regions
2. GAP REPAIR into Ab Expression Vectors



a

α

MATE

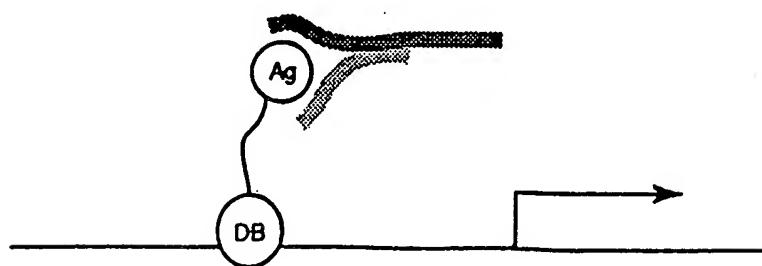


FIG. 25